

## LABORATORY ANIMAL PROJECT REVIEW

**Please note:**

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

### LAPR Information

LAPR Title: Fetal endocrine and genomic studies. Investigation of the ability of endocrine disrupting chemicals to alter reproductive development in rats

LAPR Number: 19-03-001

Principal Investigator: Exemption 6

Author of this Document: Exemption 6 /RTP/USEPA/US

Date Originated: 02/16/2016

LAPR Expiration Date: 03/31/2019

Agenda Date: 03/09/2016

Date Approved: 03/23/2016

Date Closed:

### APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	03/23/2016	DMR	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	03/23/2016	DMR	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	03/18/2016		


### Administrative Information

**1. Project Title (no abbreviations, include species):**

Fetal endocrine and genomic studies. Investigation of the ability of endocrine disrupting chemicals to alter reproductive development in rats

**Is this a continuing study with a previously approved LAPR?**

Yes

Please provide the previous LAPR# 16-03-004

**2. Programmatic Information**

**a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.**

CSS AOP (TASK 1.2C) AND SHC 2.2.2.2, Fetal endocrine and genomic studies. Investigation of the ability of Endocrine Disrupting chemicals to alter reproductive development in rats

**b. What is the Quality Assurance Project Plan (QAPP) covering this project?**

QAPP Exemption 6 2016-01

**3. EPA Principal Investigator/Responsible Employee:**

<b>Principal Investigator</b> Exemption 6	<b>Phone Number</b> Exemption 6	<b>Division</b> TAD	<b>Mail Drop</b> MD
	<b>Lotus Notes Address</b> Exemption 6 /RTP/USEPA/US	<b>Branch</b> RTB	

**4. Alternate Contact:**

<b>Alternate Contact</b> Exemption 6	<b>Phone Number</b> Exemption 6	<b>Division</b> TAD	<b>Mail Drop</b> MD
	<b>Lotus Notes Address</b> Exemption 6 /RTP/USEPA/US	<b>Branch</b>	

### SECTION A - Description of Project

**1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.**

These studies can be described as Fetal Endocrine and Genomic Studies conducted with endocrine disrupting chemicals (EDCs) including pharmaceuticals in the environment (PIEs), phthalates, and other chemicals known to disrupt gonadal steroidogenesis and/or sexual differentiation. Compounds to be tested are found in the environment in surface and waste waters and contaminated soil samples and have been shown to be hormonally active; specifically, these are thought to affect onset of puberty in girls and boys as well as reproductive development in utero. The phthalates were recently listed by the EPA as a high priority class of chemicals and our research is being used by the EPA program offices and ORD NCEA in their ongoing phthalate and cumulative risk assessment activities.

This effort is in two RAPs. The first is CSS AOP task 1.2c and the second is in the Sustainable and Healthy Communities program, Task 2.2.2.2 Health effects from early life exposures.

The CSS AOP task is to identify molecular initiating events and key events in the adverse outcome pathways (AOPs) for the androgen and estrogen signaling pathways that can be used to predict the latent, adverse effects of exposure during reproductive development in rats and/or mice. These biomarkers of effect could then be used to predict adverse effects in short-term fetal studies and reduce animal use and resource requirements since this could potentially eliminate the need for multigenerational studies.

The SHC project will identify the relationships between stressors (both chemical and non-chemical), factors (individual, family, community), and children's health and well-being. This Task will evaluate chemicals and possibly other factors (to be added by amendment) such as diet or litter size that may have an impact on pathways and stressors involved in adult adverse outcomes resulting from early life exposures.

This a continuation of a previous LAPR. In the previous LAPR we developed a fetal screening protocol that can predict which phthalates and alternatives are reproductive toxicants and which are not. We are now able to use the degree of suppression of fetal testis testosterone production and testis gene expression to predict the dose that will produce reproductive tract malformations later in life in a postnatal study. This considerably reduces the animal use and other resources needed to provide data for quantitative risk assessment on the phthalates. In addition, we have developed a phthalate AOP describing the key events related to the postnatal adverse outcomes.

In the proposed research, we are going to expand our evaluation to non-phthalate chemicals, that disrupt sexual differentiation via a diversity of molecular initiating events (MIEs), and new phthalates to determine if this also disrupt fetal testis endocrine function, to develop new AOPs for these MIEs and to integrate these AOPS with the fetal phthalate AOP to create an AOP network for the fetal androgen signaling pathway

The specific objectives of the project are

1. Identify Endocrine Disrupting Chemicals (EDCs) and mixtures which alter fetal endocrine and genomic endpoints that regulate differentiation of the reproductive tract, or that induce abnormal germ cells in the fetal testis (multinucleated germ cells, MNGs) and to investigate their fetal effects and mechanisms of toxicity.

2. In fetal animals, establish individual chemical dose-response curves, including lowest and no observed adverse effects levels (LOAEL and NOAEL) and higher dose levels, for the selected chemicals and evaluate their potency relative to known EDCs. Chemicals under investigation include phthalates, vinclozolin, ethinyl estradiol, or pharmaceuticals found in the environment (PIEs). These dose-response data will be used directly in Agency risk assessment, and inform dose selection for mixtures studies.

3. Evaluate mixtures of EDCs (pesticides, toxic substances, drugs, PIEs) and non-chemical stressors which alter testis hormone levels and gene expression or testis multinucleated germ cells (MNGs) in the fetus through identical and dissimilar mechanisms of action to determine if the effects are additive, synergistic, or antagonistic. The Food Quality Protection Act (1996) mandates that EDCs operating through identical mechanisms of action

should be assigned risk factors based on the aggregate effects of these compounds.

4. Determine if the fetal endocrine, protein, and gene expression or testis MNG measures are causally related to adverse reproductive development later in life and determine the "point of departure" (POD), i.e., the change in fetal hormone levels or gene expression resulting in an adverse effect later in life.

5. Determine the biological relevance of the fetal endocrine alterations and testis MNGs, addressing the question "How much of a change in an endpoint is required to produce a latent, adverse effect later in life".

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The proposed studies comprise three designs described below.

Dams are treated with chemicals during pregnancy, including the period of fetal sexual differentiation and euthanized during gestation so we can examine the endocrine, physiological, genomic, and proteomic alterations from the tissues of fetal animals. The dams will be euthanized during gestation, before any litters are born.

## **2. Scientific rationale for proposed animal use.**

### **a. Why is the use of animals necessary?**

Whole animal studies are essential to determine the effects of EDCs on development as no suitable alternative method or model is available that accounts for the maternal-placental-fetal interactions that are essential for human health risk assessment.

### **b. Justify the species requested:**

Rats are some of the best understood animal models for study of the potential effects of chemicals on human reproductive development and function. The reproductive physiology, endocrinology, and development of the rat has been studied for over 80 years and is the only species used in the EPA's multigenerational reproductive toxicity test and the proposed endocrine screening assays. Hence, we must use rats in our studies in order for our results to be relevant for EPA risk assessments and the endocrine screening program.

## **3. How was it determined that this study is not unnecessary duplication?**

We evaluate all of the published scientific literature on each chemical and, when possible, examine the study files submitted by industry to the regulatory agencies (information may be available on the EPA, Food and Drug Administration, or World Health Organization websites or regulations.gov). We searched PUBMED and government risk and hazard assessment documents by chemical name and CAS number to be sure the proposed studies are not duplicative.

## **SECTION B - In Vivo Procedures**

### **1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.**

Dams are treated with chemicals during pregnancy, including the period of fetal sexual differentiation and euthanized during gestation so we can examine the endocrine, physiological, genomic, and proteomic alterations from the tissues of fetal animals. Dosing periods and necropsy times are specified below for each study type. Ninety-day old dams are typically shipped from the breeder on gestation day (GD) 2. Chemical names for the abbreviations are listed in section D.

Dose levels are selected to provide a dose range over 1 to 2 log units. The top dose, well below the LD50, is expected to produce significant developmental effects without causing maternal toxicity and the lowest dosage level is expected to not produce any developmental effects.

#### **Design 1. Single-Chemical Dose-Response Studies.**

Determine dose-response curve for individual chemical's effects on fetal insl3 levels, testicular T production, and gene expression.

Dosing once per day on GD 14-18; five dose levels (including control).

Necropsy dams on GD 18 to measure fetal hormones and gene expression.

This is conducted over two blocks (15 dams per block).

6 dams/group x 5 dose levels = 30 dams per chemical

Design 2. Single-Chemical Studies to determine the ability of individual chemical's effects on fetal testis Multinuclear Germ Cells (MNGs).

Run six blocks of up to 16 dams per block (up to 3 chemicals and a vehicle control; n=4/group)

Dosing once per day on GD 14-20; one dose level per chemical.

Necropsy dams on GD 20, to save testes for histological determination of MNGs.

Design 3. Mixture Dose-Response Studies

Evaluate dose-additivity of multiple chemicals and screen for changes in male fetal gene expression.

Multiple chemicals administered in mixture on GD 14-18 with a necropsy on GD 18. We will prepare dosing formulations of multiple chemicals such that each chemical, based on individual dose-response curves, will contribute equally to the potential effect on sexual differentiation. In a given study, each treatment group will represent a different dilution of the mixture; i.e., the chemical proportions will be held constant (a fixed-ratio dilution), with each chemical contributing equally to the potential effect on sexual differentiation based upon the relative potency of each individual chemical.

The top dose will be selected to not be maternally toxic based upon previous experience with each individual chemical and similar mixture studies. Each chemical will be administered in the top dose group at one-fifth of the Lowest Observed Adverse Level (LOAEL) for developmental reproductive toxicity. The dose groups will be about 0, 12.5%, 25%, 50% and 100% of the top dose. (The chemicals and the top dose level are shown in the attached table.)

**2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.**

The numbers of animals requested per group is the minimum number required to give statistical significance for the parameters of interest. We have found that six litters per treatment group generally provides us with robust statistical outcomes. In cases where it is deemed necessary to pursue low-dose (subtle) effects, additional animals and larger group sizes may be required; if such need arises, an amendment will be submitted. 90 day old dams are shipped on GD 7 and given at least one week to acclimate to the facility.

Design 1:

6 chemicals x 6 dams/group x 5 dose levels = 180 dams

(PARACETAMOL, CLOFIBRATE, Bisphenol C, FINASTERIDE, FLUTAMIDE, DEXAMETHASONE)

Design #2 - seventeen chemicals and the vehicle control

17 chemicals x 1 dosage/chemical x 4 dams/dosage = 68 treated dams

6 blocks x 4 control dams/block = 24 control dams

Total for Design 2: 68 treated + 24 control = 92 dams

Chemicals include

Block 1: DPEP, DEP, DEHP, vehicle

Block 2: DIBP, DCHP, DOTP, vehicle

Block 3: DMP, VINCLOZOLIN, LINURON, vehicle

Block 4: DEXAMETHASONE, Bisphenol C, DBP, vehicle

Block 5: DIBP, TETRABROMO DEHP, vehicle

Block 6: Bisphenol S, Bisphenol A, pyrifluquinazon (PFQ) and the vehicle

Design 3:

1 mixture (with 18 chemicals) x 9 dams/group x 5 dose (dilutions of the top dose) levels = 45 dams

Run in 3 blocks with 15 dams/block.

Overall:

180 (Design 1) + 92 (Design 2) + 45 (Design 3) = 317 dams total

**3. State how many animals over the study period are expected to be used under the following three categories**

of pain/distress (USDA nomenclature as defined in the instructions ): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	317	0
D) Potential pain/distress relieved by appropriate measures:	0	0
E) Unrelieved pain/distress:	0	0

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes) ☐ Survival surgery  
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

For assessments of male fetuses, oral (gavage) daily dosing of dams will be on GD 14-18 (for endocrine measures or GD 14-20 (for MNG enumeration).

We generally use corn oil as the vehicle for chemicals not soluble in water, and water is the vehicle for water-soluble chemicals. Unless poorly soluble, the same dosing volume (2.5 ml/kg) will be used for both vehicles. For poorly soluble chemicals, the dosing volume will not exceed 5 ml/kg.

For single-chemical dose-response studies, the dose-ranges will be determined from previous work and the literature prior to the study, but will not exceed dosages listed in D1.

b. Survival Blood Collections (method, volume, frequency):

none

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

none

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

none

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:

Animals will be examined upon arrival and identified by cage cards with unique numbers. They are examined and weighed daily during dosing to necropsy by lab managers **Exemption 6** **Exemption 6** another qualified technician, a postdoctoral trainee, and/or PI on the study. Personell are listed in Section E1.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

none

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

none

c. Testing methods:

none

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

none

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

none

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

none

**7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)**

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

na

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

na

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

na

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

na

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

☐ Yes ☒ No

f. Identify any surgical procedures performed at other institutions or by vendors:

none

**8. Humane interventions (for treatments/procedures in all categories).**

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

We do not anticipate that any of the treatments will produce any adverse maternal effects. If overt maternal toxicity (e.g., deteriorating body condition, rough hair coat, lethargy, abnormal behavior) is observed, dosing will be immediately terminated and animals will be immediately euthanized if warranted. The Attending Veterinarian may be consulted as warranted. When complications (e.g., vaginal bleeding) are noted during pregnancy the animals are dealt with humanely and euthanized if necessary. If problems are noted we would like to continue to be notified immediately by email **Exemption 6** so that the situation can be dealt with promptly.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

Deteriorating body condition, rough hair coat, lethargy, wounds, abnormal behavior, dystocia, or any other sign of distress would result in removal of animals from the study.

**9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.**

na

## **SECTION C - Animal requirements**

**Describe the following animal requirements :**

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	287
b. Animals to be transferred from another LAPR:	30
LAPR Number that is the source of this transfer:	16-03-004
c. Animals to be transferred from another source:	0
d. Offspring produced onsite (used for data collection and/or weaned):	0
e. TOTAL NUMBER of animals for duration of the LAPR	317

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Sprague Dawley  
Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)  
none

4. Sources of animals:  
Charles River Laboratory or Envigo (formerly Harlan) Laboratory

5. Provide room numbers where various procedures will be performed on animals:  
Exemption 6  
Exemption 6  
Exemption 6 (our laboratory) and the animal rooms (dosing and weighing only)

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.  
no Room Numbers:

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)  
none

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

Timed-pregnant animals need to be individually housed in newer, clear plastic cages with heat-treated pine shavings as bedding upon receipt. No environmental enrichment since most of these products have not been adequately tested for endocrine disrupting chemicals and contain unknown concentrations of the toxic chemicals (plastics, plasticizers, metals, etc.) we are studying. Cotton products have been shown to have dioxin-like activity, and plastic devices have been repeatedly shown to contain phthalates, bisphenols and/or other endocrine active substances. Devices and animal beddings made from recycled paper are known to have very high concentrations of phthalates (references attached)

Because these studies are intended for comparison with postnatal studies (LAPR 16-04-002), where dams must be singly housed to maintain litter identities, the dams in the current studies must also be singly housed so the environmental and social factors are identical in the two types of studies.

NIH 07 diet during gestation.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

#### **10. Housing and Enrichment.**

***The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.***

We will only accept nesting material after assurance that it is not made of recycled paper, or has a plastic or phthalate coating and has been shown to be free of contaminants like BPA, phthalates or other plasticizers, pesticides, and metals as these might confound low-dose studies by exposing the controls to these chemicals.

### **SECTION D - Agents Administered to Animals**

***1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used. Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.***

Compounds to be or that may be tested, the maximum dose, and the oral LD50 are as follows:

Flutamide, 100 mg/kg/d, acute oral LD50 is 787 mg/kg/d

Finasteride, 100 mg/kg/d, acute oral LD50 418 mg/kg

BPC = Bisphenol C (CAS 14868-03), 400 MG/KG/D; oral LD50 = 980 MG/KG  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

BPA = Bisphenol A, 800 mg/kg; LD50=4-12 g/kg rat  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

BPS = Bisphenol S, 800 mg/kg/d; LD50 (oral rat) 4556 mg/kg

PFQ= Pyrifluquinazon, 150 mg/kg. Oral LD50 in female rat: >300 and <2000 mg/kg body weight

DBP= Dibutyl Phthalate 1500 mg/kg; Rat oral LD 50 = 8000 mg/kg

DEHP= Bis (2-Ethylhexyl) Phthalate - 1500 mg/kg; Rat oral LD 50 = 30,000 mg/kg

BBP=Benzybutyl Phthalate - 1500 mg/kg; Rat oral LD 50 = 2330 mg/kg

DiBP=Diisobutyl phthalate 1000 mg/kg; LD50 = 15,000mg/kg

DHeP= DiHeptyl phthalate 1000 mg/kg; LD50=>2 gms/kg  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

DiHP= Diisooheptyl phthalate 1000 mg/kg; LD50= >2 gms/kg

DPeP= Dipentyl phthalate 600 mg/kg; LD50> TDLo = 2 gms/kg

DHP=Dihexyl phthalate 1000 mg/kg; LD50=30 g/kg

DINP=Di-iso-nonyl phthalate, 1000 mg/kg, LD50/rat: > 5000 mg/kg

DEP= Diethyl phthalate 900mg/kg; LD50=8200mg/kg

DMP= Dimethyl phthalate 1000mg/kg; LD50=6200mg/kg

DnOP=di-n-octyl phthalate, 1000 mg/kg, Oral LD50: 30,000 mg/kg

Vinclozolin - 300 mg/kg; Rat oral LD 50 = 10,000 mg/kg

Procymidone - 300 mg/kg; Rat oral LD 50 = 7000 mg/kg

Prochloraz - 250 mg/kg; Rat oral LD 50 = 2500 mg/kg  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

Linuron - 200 mg/kg; Rat oral LD 50 = 1500 -4000 mg/kg

DCHP = Dicyclohexyl phthalate; 1000 mg/kg; RAT ORAL LD 50 > 5000 MG/KG

Dexamethasone - 250 mg/kg; Rat oral LD50 = 7.5 g/kg  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

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DPP = di-propyl phthalate, 1 g/kg ; Oral, rat LD50: 7800 mg/kg  
Included in HSRP 131. "Investigation of synergistic effects of estrogens".

DPHP = di-propyl heptyl phthalate, 1.5 g/kg; Oral rat LD50> 2 g/kg

DnHP = Di-n-Heptyl phthalate 1000 mg/kg; LD50=>2 gms/kg DHeP = Dihexyl phthalate 1000 mg/kg;  
LD50=30 g/kg

DnOP = di-n-octyl phthalate, 1000, ORAL (LD50): Acute: 30000 mg/kg WY-14,643, 200 mg/kg; Rat oral LD50: 1050 mg/kg

DIDP = diisodecyl phthalate, 1.5 g/kg/d; rat oral LD50 >6 g/kg

Clofibrate - 400 mg/kg/d; rat oral LD50 >900 mg/kg (listed as 910, 940, and 1220 on different MSDS sheets)

Paracetamol - 500 mg/kg/d; rat oral LD50 = 1944 mg/kg.

Corn oil (food grade for oral gavage), within 1 yr of receipt. 5 ml/kg. LD50: Oral [Rat] >100 ml/kg,

**2. Describe compounds to be administered to animals.**

**a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.**

All drugs will be pharmaceutical grade (paracetamol, clofibrate, dexamethasone, finasteride and flutamide). Non-drug chemicals will the highest purity available since pharmaceutical grades do not exist

**b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.**

none

**c. Provide a statement regarding any safety precautions necessary for handling any of these materials.**

All necessary PPE (lab coats, safety glasses, gloves) during dose preparation, dosing and necropsy.

**NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.**

## SECTION E - Personnel Training and Experience

**1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.**

**Use this area to type in additional personnel information not available in the table drop-down lists:**

**Hint:** The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	design, dosing, necropsy	30 plus years of experience and all NHEERL-required training
Exemption 6	Technical Staff	dosing, necropsy	5 plus years of experience and all NHEERL-required training
Exemption 6	Technical Staff	design, dosing, necropsy	20 plus years of experience and all NHEERL-required training
Exemption 6	Technical Staff	necropsy	several years plus years of experience all NHEERL-required training and work with domestic animals
Exemption 6	Post-Doc	design, dosing, necropsy	over one year experience, all NHEERL-required training, and will be trained by experienced technical staff and PI
Exemption 6	Technical Staff	necropsy	20 plus years of experience and all NHEERL-required training
Exemption 6	Associate Principal Investigator	necropsy	20 plus years of experience all NHEERL-required training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

## SECTION F - Animal Breeding Colonies

**This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.**

**Describe:**

- 1. Estimated number of breeding pairs and liveborn per year** 0
- 2. Breeding protocols and recordkeeping**
- 3. Methods for monitoring genetic stability**
- 4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR**

## **SECTION G - Euthanasia**

**1. When will the animals be euthanized relative to experimental procedures?**

Dams and fetuses will be euthanized on GD 18 or GD 20, the day of the last dose

**2. Describe the euthanasia techniques:**

**Method(s):** Decapitation

**Agent(s):** Backup guillotine will be available.

**Dose (mg/kg):**

**Volume:**

**Route:**

**Source(s) of information used to select the above agents/methods:**

- Veterinary Staff, 2013 AVMA Guidelines on Euthanasia.

**3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).**

compliant with 2013 AVMA Guidelines

**4. Describe how death is to be confirmed.**

decapitation

## **SECTION H - Disposition of Used and Unused Animals**

**Describe the disposition of any animals remaining after project completion.**

Euthanized by Animal Care Contractor

**The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?**

☒ Yes ☐ No

## **SECTION I - Assurances**

**1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.**

**2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.**

**3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.**

**4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.**

**5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.**

**6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.**

**7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.**

8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

<b>EPA Principal Investigator</b>	<b>Certification Signature Date</b>
Exemption 6 Exemption 6	02/16/2016

Submitted: 02/29/2016

### Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	Approval Date	Phone Number	Division	Mail Drop
Exemption 6	03/01/2016	Exemption 6 Lotus Notes Address	TAD Branch	MD Submitted to Branch Chief for Approval
	by Exemption 6 Exemption 6 Exemption 6 TP/USEP A/US	Exemption 6 Exemption 6 Exemption 6 RTP/USEP A/US	RTB	02/29/2016 02:48 PM

### ATTACHMENTS



Ahr activation by rodent enrichment devices Tischkau and Mukai 2009.pdf



waste paper recycling Critical substances Pivnenko et al 2015.pdf



lab animal issues in the study of EDCs everitt and foster 2004 mentions bedding and enrichment devices.pdf



kondo et al 2010 phthalates in rodent diets and bedding.pdf



18 chemical mixture study in FPS chemicals and doses for LAPR .docx

### Actions

First Update notification sent: 01/31/2017  
 Second Update notification sent: 03/02/2017  
 First 2nd Annual notification sent:  
 02/02/2018  
 Second 2nd Annual notification sent:  
 03/02/2018  
 1st Expiration notification sent:  
 2nd Expiration notification sent:

### History Log:

